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Allostimulatory analysis of a newly-defined and widely-distributed Mls superantigen

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Abstract. We previously noted that $Mls^{a,c}$ C58/J responder cells proliferated unexpectedly to H-2^k-compatible Mls^a or Mls^c prototypic stimulator cells in a primary mixed lymphocyte reaction. The present investigation was performed to evaluate whether the response of C58/J T cells to these H-2- and Mls-compatible stimulator cells could functionally identify a newly-defined member of the Mls superantigen family through its allostimulatory ability. We observed that C58/J responder cells also proliferated when cultured with H-2-compatible prototypic Mls^{null}, Mls^b (nonstimulatory), or $Mls^{a,c}$ splenic stimulator cells. The widely distributed nature of the non-MHC ligand recognized by C58/J T cells is indicated by the finding that 11 of 12 H-2^k inbred mouse strains clearly expressed this specificity. A gradient of stimulatory capacity from low to high across this newly-defined non-MHC difference was detected with splenocytes from these different inbred mouse strains. The $Mls^{a,c}$ genetic composition of C58/J was confirmed by the observation that crossing C58/J with parental B10.BR (responsive to both Mls^a and Mls^c determinants) generated F_1 progeny that were unresponsive to H-2^k-compatible Mls^a, Mls^c, or $Mls^{a,c}$ stimulator cells. Like prototypic Mls^a and Mls^c, the non-MHC specificity recognized by C58/J responder cells, termed Mls^f, was particularly sensitive to radiation (versus mitomycin C) treatment of the stimulator cells, was greatly augmented after anti-IgD activation of splenic stimulator cells, was blocked with anti-MHC class II antibody, and was effectively presented by phenotypically normal female but not B cell-defective xid^+ male CBA/N F_1 stimulator cells.

Introduction

Mouse minor lymphocyte stimulating (Mls) determinants were first identified by the capacity of stimulator populations expressing these antigens to trigger proliferation of H-2-compatible responder T cells in a primary mixed lymphocyte reaction (MLR; Festenstein 1970, 1973). These non-major histocompatibility complex (MHC) moieties stimulated both $CD4^+$ (Janeway et al. 1980) and $CD8^+$ (Webb and Sprent 1990) T cells in vitro with a high precursor frequency (Janeway et al. 1980; Miller and Stutman 1982).

More recently the important role of Mls antigens in shaping the T cell repertoire has received considerable attention. In mice expressing Mls^a as a self-antigen, mature thymocytes and peripheral T cells were depleted of those populations that expressed $V\beta6^+$ (MacDonald et al. 1988), $V\beta8.1^+$ (Kappler et al. 1988), or $V\beta9^+$ (Happ et al. 1989; Vacchio and Hodes 1989) T cell receptor (Tcr) segments. In mice expressing Mls^c, T cells that bear the $V\beta3$ (Abe et al. 1988; Pullen et al. 1988, 1989) segment were largely depleted. The *MHC* haplotype of the mouse strain also influenced this process of intra-thymic clonal deletion of self-reactive T cells mediated by Mls antigen (Kappler et al. 1988; Pullen et al. 1988). The high precursor frequency of Mls responsive T cells observed earlier in the primary MLR (Janeway et al. 1980; Miller and Stutman 1982) correlated with the finding that T cell expression of $V\beta6$, $V\beta8.1$, and $V\beta9$ (Kappler et al. 1988; MacDonald et al. 1988; Happ et al. 1989) or $V\beta3$ (Abe et al. 1988; Pullen et al. 1988, 1989) was associated with T cell reactivity to Mls^a or Mls^c, respectively. Other α or β chain segments of the Tcr did not appear to contribute to specificity in the T cell response to these Mls antigens (Abe et al. 1988; Kappler et al. 1988; Pullen et al. 1988).

In the first demonstration that self-tolerance can be generated by clonal elimination of self-reactive T cells during development in the thymus, the deletion of $V\beta17a^+$ peripheral T cells and mature thymocytes in mice expressing MHC class II H-2E molecules was described (Kappler et al. 1987). This deletion probably resulted from self-tolerance induction, since most random

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Vβ17a$^+$ T cell hybridomas were responsive to E$^+$ splenocytes, B cell lymphomas, and B cell hybridomas (Kappler et al. 1987). Further studies showed that an undefined superantigen expressed by B cells (but not other E$^+$ cell types) was recognized in association with the E molecule by Vβ17a$^+$ T cells (Marrack and Kappler 1988). E-expressing mouse strains were also found to eliminate Vβ5.2$^+$ (Woodland et al. 1990), Vβ7$^+$ (Vacchio and Hodes 1989), and Vβ11$^+$ (Bill et al. 1988, 1989; Vacchio and Hodes 1989) T cells; undefined non-*MHC* gene products also influenced this deletion process (Bill et al. 1988; Vacchio and Hodes 1989; Woodland et al. 1990). In confirming and extending the range of documented Vβ deletions in the T cell repertoire of inbred mice, substantial decreases in expression of Vβ3, 5, 6, 7, 9, 11, 12, and 16 due to self-determinants were observed (Vacchio and Hodes 1989). In this study, C58/J animals, unlike other E$^+$ animals, showed no quantitative decrease in expression of Vβ11- and Vβ12-bearing T cells in their periphery. Non-MHC ligands not encoded in C58/J mice but expressed in other mouse strains were subsequently demonstrated to delete T cells expressing either of these two segments of the Tcr (Vacchio et al. 1990).

In studying nonprototypic mouse strains as responders and stimulators in an Mls-defined MLR (Ryan et al. 1990a), this laboratory observed additional intriguing properties associated with C58/J lymphocytes. As stimulator cells, C58/J splenocytes presented the Mls^c determinant (formerly considered a weak-to-intermediate stimulatory moiety) in an autosomally dominant superstimulatory form. In addition, although considered genotypically Mls^{a,c}, C58/J responder T cells proliferated vigorously to H-2^k-compatible Mls^a or Mls^c prototypic stimulator cells. Studies of Vβ usage and deletion in inbred mice have provided clues that undefined self-deleting superantigens exist that are distinct from Mls^a and Mls^c (Marrack and Kappler 1988; Bill et al. 1989; Vacchio and Hodes 1989; Woodland et al. 1990). Thus the purpose of the present investigation was to evaluate the possibility that the unanticipated proliferative response of naive C58/J T cells to H-2 and Mls-compatible stimulator cells observed in earlier studies (Ryan et al. 1990a; Vacchio et al. 1990), could functionally identify a newly-defined member of the Mls superantigen family through its allostimulatory capacity.

Materials and methods

Animals. AKR/J (*H-2^k*, *Mls^a*), BALB/c (*H-2^d*, *Mls^c*), (BALB/c × DBA/2)F$_1$ (*H-2^d*, *Mls^{a,c}*), B6.AKR-H-2^k/FlaEg (*H-2^k*, *Mls^b*), B10.BR (*H-2^k*, *Mls^b*), B10.D2 (*H-2^d*, *Mls^b*), CBA/CaJ (*H-2^k*, *Mls^b*), CBA/J (*H-2^k*, *Mls^{a,c}*), CBA/N (*H-2^k*, *Mls^{null}*), CE/J (*H-2^k*, *Mls^{a,c}*), C2H/HeJ (*H-2^k*, *Mls^c*), (C57BL/6 × DBA/2)F$_1$ (*H-2$^{b/d}$*, *Mls^{a,b,c}*), C57BL/10Sn (*H-2^b*, *Mls^b*), C57BR/cdJ (*H-2^k*, *Mls^b*), C58/J (*H-2^k*, *Mls^{a,c}*), MA/My (*H-2^k*, *Mls^a*), RF/J (*H-2^k*, *Mls^{a,c}*), SJL/J (*H-2^s*, *Mls^s*) were obtained from the Jackson Laboratory, Bar Harbor, ME. (B10.BR × CBA/J)F$_1$ (*H-2^k*, *Mls^{a,b,c}*), (B10.BR × C58/J)F$_1$ (*H-2^k*, *Mls^{a,b,c}*), (B10.D2 × C58/J)F$_1$ (*H-2$^{d/k}$*, *Mls^{a,b,c}*), (C57BL/10 × C58/J)F$_1$ (*H-2$^{b/k}$*, *Mls^{a,b,c}*), (CBA/CaJ × C58/J)F$_1$ (*H-2^k*, *Mls^{a,b,c}*), (CBA/N × AKR/J)F$_1$ (*H-2^k*, *Mls^{null,a}*), (CBA/N × B10.BR)F$_1$ (*H-2^k*, *Mls^{null,b}*), (CBA/N × CBA/CaJ)F$_1$ (*H-2^k*, *Mls^{null,b}*), and (DBA/2 × C58/J)F$_1$ (*H-2$^{d/k}$*, *Mls^{a,c}*) mice were bred at the Naval Medical Research Institute (NMRI) with parental breeding stock purchase from The Jackson Laboratory Bar Harbor, ME. BALB.K (*H-2^k*, *Mls^c*) and B10.Q (*H-2^q*, *Mls^b*) mice were kindly provided by Drs. Florence Rollwagen and Walter Weiss (NMRI), respectively. Because it is likely that both *Mls^a* (Click et al. 1982; Ryan et al. 1990b) and *Mls^c* (Click and Adelmann 1988; Abe et al. 1989; Pullen et al. 1989) are the products of multiple genes, the original small case letter designations for Mls specificities have been utilized throughout this study as collective symbols for these goups of multi-gene products. CBA/N mice have been designated *Mls^{null}* rather than (nonstimulatory) *Mls^b* to indicate that their X-linked B cell defect usually precludes effective in vitro presentation of Mls determinants (Ahmed and Scher 1976). Experiments were performed with 8–20-week-old animals that were maintained in filtered-air isolators in the animal colonies of the Naval Medical Research Institute.

Mixed lymphocyte reaction. Responder cells for the MLR were obtained by enriching for T cells by passage of splenocytes over a nylon wool column (Julius et al. 1973). Unprimed responder T cells were cultured at the density of 3×10^5 cells/microtiter well, usually with $1.0 - 9 \times 10^5$ stimulator cells in a total volume of 0.2 ml. The MLR culture medium consisted of RPMI 1640 (Hazelton Laboratories, Lenexa, KA) with gentamicin (50 µg/ml), L-glutamine (2m*M*), HEPES buffer (25 m*M*), 5% fetal calf serum FCS; Hyclone, Ogden, UT), and 5×10^{-5} *M* 2-mercaptoethanol. Each MLR was performed with quadruplicate cultures in round-bottomed microculture plates (No. 3799; Costar, Cambridge, MA) and was maintained in a humidified atmosphere of 5% CO_2 at 37 °C. The culture was harvested after 72–96 h onto glass fiber filter paper after a 12 h pulse with 1 µCi (37KBq)/microtiter well of [^{3}H]TdR (specific activity = 2 Ci/m*M*; New England Nuclear, Boston, MA). Incorporated [^{3}H]TdR was measured on a Beckman scintillation spectrometer. The results were calculated from uptake of [^{3}H]TdR and are expressed as the arithmetic mean in cpm of triplicate or quadruplicate cultures. The standard errors were generally less than 10% of the mean. The statistical significance of the mean cpm of each experimental group was calculated with Student's *t* test. Mean differences were considered to be significant when $P < 0.05$. The monoclonal IA^k-specific antibody, 10-362, and the E-specific antibody, 14-4-4, that were used in MLR blocking experiments were kindly provided by Dr. Ada Kruisbeek (NIH, Bethesda, MD). The monoclonal K^k-specific antibody, 11-4.1 (#1320, Becton Dickinson, San Jose, CA), was also used in the MLR blocking studies.

In vivo anti-IgD treatment and in vitro preparation of MLR stimulator cells. Recipient mice were injected intravenously with 100 µg affinity-purified goat antimouse IgD (GaMD) reagent in a volume of 0.2 ml that

was prepared as previously described (Finkelman et al. 1981). Twenty-four hours later, the spleens were removed and gently teased in Hanks' balanced salt solution (HBSS) with 10% fetal calf serum (FCS), then irradiated with 3000 R (^{137}Cs) after the removal of the red blood cells with a hypotonic lysing buffer, washed again, and resuspended in the MLR medium described above. This laboratory has shown that in vivo (Ryan et al. 1983, 1987a, 1987b, 1988) or in vitro (Ryan et al. 1988) activation of splenic B cells with GaMD prior to irradiation substantially enhances the capacity to present both Mls^a and Mls^c specificities. When mitomycin C (85,549.9; Aldrich Chemical Co., Milwaukee, WI) was used instead of irradiation to prevent normal or GaMD-activated splenic stimulator cells from dividing in the primary MLR, the splenocytes (1×10^7/ml) were suspended in HBSS and exposed to mitomycin C (75 µg/ml) for 30 min at 37 °C in the absence of light; they were than washed three times before being added to the responder T cells.

T cell depletion of splenocyte populations. Spleens were gently teased in RPMI 1640 plus 1% FCS, depleted of red blood cells with ammonium chloride lysing buffer, washed twice, and filtered through sterile nylon mesh (HC3-110; Tetco Inc., Elmsford, NY) to remove tissue clumps. The single cell preparation was then suspended in monoclonal anti-Thy 1.2 ascites fluid (NEI-001; New England Nuclear Research Products, Boston, MA) at 1:500 dilution or (for Thy 1.1/1.2 F_1 animals) a cocktail of anti-Thy 1.1 (NEI-002; New England Nuclear Research Products, Boston, MA) plus anti-Thy 1.2 at 1:500 dilution for half an hour at 4 °C. The treated cells were then washed twice and resuspended in a 1:8 dilution of rabbit complement (ACL 3051: Low-Tox M, Accurate Chemical & Scientific Corp., Westbury, NY) for 45 min at 37 °C. The remaining splenocytes were washed twice and refiltered through sterile nylon mesh to remove dead cells and tissue debris before treatment with mitomycin C.

Results

The Mls^{a,c} prototypic mouse strain, CBA/J, elicits unidirectional proliferation of H-2 and Mls-compatible C58/J. Previous studies with heterogeneous (Ryan et al. 1990a) or cloned (Abromson-Leeman et al. 1988a, 1988b) T cells have indicated that C58/J stimulator cells express both Mls^a and Mls^c determinants. Therefore, it is consistent with self-tolerance that the mature peripheral T cell pool of C58/J is deleted of populations that express Vβ6 and Vβ9 (associated with responsiveness to *Mls^a*) and Vβ3 (associated with responsiveness to Mls^c; Vacchio and Hodes 1989). Nevertheless, it was recently shown that C58/J responder cells proliferated at very detectable levels when cultured with H-2-compatible prototypic Mls^a (AKR/J) and Mls^c (C3H/HeJ) stimulator cells (Ryan et al. 1990a; Vacchio et al. 1990). Since the latter result could be interpreted as conflicting with the other findings that suggested C58/J encodes Mls^a and Mls^c, we explored the non-MHC polymorphic similarities and differences between C58/J and the Mls^{a,c} prototypic strain, CBA/J, in a primary MLR (Fig. 1).

As expected, both C58/J and CBA/J splenocytes were capable of triggering vigorous proliferation of H-2^k-compatible Mls^b B10.BR T cells across the Mls^a and Mls^c specificities that have been reported to be expressed by both of these stimulator populations (Fig. 1, left panel). While CBA/J T cells remained unresponsive to H-2^k and

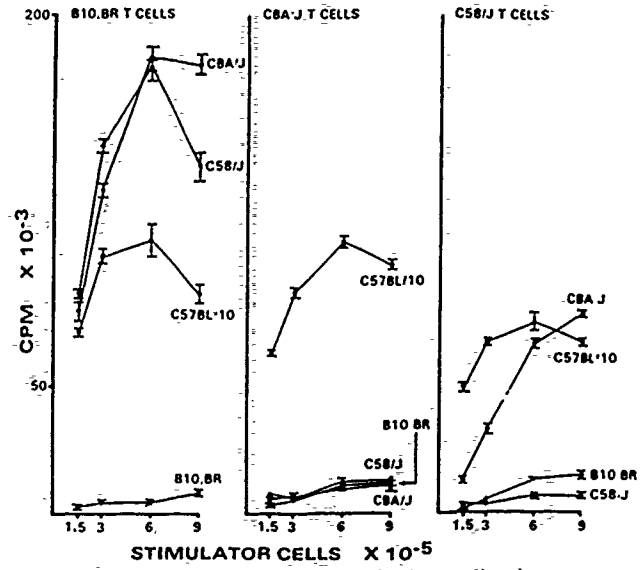

Fig. 1. Comparison of mutual MLR stimulatory capacity among H-2^k-compatible B10.BR, CBA/J, and C58/J splenocytes. Nylon wool-purified splenic T cells (3×10^5/well) from B10.BR (Mls^b), CBA/J (Mls^{a,c}), or C58/J (Mls^{a,c}) strains were cultured with normal mitomycin C-treated splenocytes from these H-2^k-compatible animals as well as from H-2-different C57BL/10 (H-2^b) mice, at the concentrations shown. The MLR culture was harvested after 72 h.

Mls^{a,c}-compatible mitomycin C-treated C58/J splenocytes (Fig. 1, center panel), C58/J T cells proliferated unambiguously in a dose-dependent manner to CBA/J stimulator cells (Fig. 1, right panel). Indeed, C58/J T cells also appeared to respond at a lower level to H-2-compatible Mls^b (so-called nonstimulatory) B10.BR splenocytes.

To test the functional capacity of parental C58/J to induce self-tolerance in F_1 progeny to prototypic Mls^a and Mls^c determinants, H-2^k-compatible Mls^b B10.BR animals that are fully responsive to Mls^a and Mls^c were crossed with C58/J. B10.BR was also crossed with Mls^{a,c} CBA/J; the resulting F_1 was previously shown by this laboratory to be specifically unresponsive to Mls^a (AKR/J) and Mls^c (C3H/HeJ) stimulator cells (Ryan et al. 1987). We observed that both (B10.BR × C58/J)F_1 and (B10.BR × CBA/J)F_1 T cells were poorly responsive to prototypic Mls^a AKR/J, Mls^c C3H/HeJ, and Mls^{a,c} (C3H × AKR)F_1 mitomycin C-treated splenocytes (Table 1). The lack of response of (B10.BR × CBA/J)F_1 T cells to C58/J stimulator cells was in agreement with these results and the previous finding with heterogeneous (Ryan et al. 1990a) and cloned (Abromson-Leeman et al. 1988a, 1988b) T cells that C58/J expresses both Mls^a and Mls^c determinants. A similar conclusion could be reached with the observation that (B10.BR × C58/J)F_1 T cells did not vigorously proliferate to the prototypic Mls^a, Mls^c, and Mls^{a,c} stimulator cells.

However, because C58/J T cells could respond to H-2-compatible prototypic Mls^a and Mls^c (Ryan et al.

Table 1. Like Mls^{a,c} CBA/J, parental C58/J induces self-tolerance in F_1 progeny to prototypic Mls^a and Mls^c determinants.

Stimulator cells*			Responder cells†		
Strain	H-2	Mls	B10.BR	(B10.BR × CBA/J)F_1	(B10.BR × C58/J)F_1
Syngeneic	k	–	3,196‡	6,123	5,898
AKR/J	k	a	128,414	7,607	10,589
C3H/HeJ	k	c	20,885	6,388	5,703
(C3H × AKR)F_1	k	a, c	102,990	5,275	4,401
CBA/J	k	a, c	203,068	8,016	7,957
C58/J	k	a, c	184,580	6,237	5,995
C57BL/10	b	b	92,132	97,599	82,510

* Stimulator cells were normal (nonactivated) splenocytes (9×10^5/well) treated with mitomycin C.
† Responder cells were nylon wool-purified splenic T cells (3×10^5/well).
‡ Values are cpm of mean arithmetic [^{3}H]TdR uptake of quadruplicate cultures, harvested at 72 h. *Underlined numbers* are those that are significantly greater ($p < 0.05$) than responses to syngeneic stimulators.

Table 2. The presentation of the non-MHC specificity recognized by C58/J T cells, like prototypic Mls^a and Mls^c, is impaired by irradiation and substantially augmented by stimulator pretreatment with GaMD antibody.

Stimulator cells*				Responder cells†		
H-2^k strain	Mls	Inactivation treatment	GaMD treatment	B10.BR (Mls MLR)	C58/J (non-H-2 MLR)	(BALB/c × DBA/2)F_1 (H-2 MLR)
Experiment 1						
Syngeneic		M	–	3,838‡	6,466	10,318
		R		2,338	4,983	7,055
AKR/J	a	M	–	148,217	43,306	124,156
		R		17,218	5,082	65,684
CBA/CaJ	b	M	–	7,532	106,388	173,988
		R		3,632	25,536	103,182
C3H/HeJ	c	M	–	28,400	18,050	147,702
		R		7,243	4,128	76,479
CBA/J	a, c	M	–	213,097	77,565	173,942
		R		53,930	13,907	98,842
CBA/N	null	M	–	6,788	40,036	135,020
		R		2,380	5,209	63,060
Experiment 2						
Syngeneic		R	–	3,029	4,958	
			+	3,764	9,095	
AKR/J	a	R	–	24,807	9,692	
			+	261,103	64,723	
CBA/CaJ	b	R	–	2,747	9,726	
			+	2,993	180,606	
C3H/HeJ	c	R	–	12,628	4,952	
			+	48,685	40,423	
CBA/J	a, c	R	–	50,013	14,647	
			+	398,845	280,338	
CBA/N	null	R	–	4,083	10,942	
			+	5,148	17,603	

* Stimulator cells were normal (nonactivated) splenocytes (6×10^5/well) that were irradiated (R) with 3000 R or mitomycin C (M)-treated (Experiment 1) or normal or GaMD-activated splenocytes (6×10^5/well) that were irradiated (R) with 3000 R (Experiment 2).
† See legend to Table 1.
‡ See legend to Table 1.

1990a; Vacchio et al. 1990; see below) as well as prototypic Mls^{b} and Mls^{a,c} splenocytes (Vacchio et al. 1990; Fig. 1), one could also conclude from the latter observation that a self-determinant expressed by Mls^{b} (non-stimulatory) B10.BR induced self-tolerance in the C58/J parental component of (B10.BR × C58/J)F_1 animals to each of the prototypic Mls stimulator cells listed in Table 1.

The specificity recognized by C58/J T cells on H-2^{k}-compatible stimulator cells is sensitive to irradiation and is augmented by GaMD exposure. The above results suggested that C58/J T cells were recognizing a newly-defined non-MHC MLR specificity that was co-expressed with previously defined Mls specificities on prototypic Mls presenting cells. To explore the functional similarity of this non-MHC antigen to prototypic *Mls* determinants, we wished to test the sensitivity of this moiety to irradiation and the effect of GaMD pretreatment on presenting capacity. It has previously been shown that irradiation of splenocytic stimulator cells substantially inhibits their capacity to present prototypic Mls^{a} (Webb et al. 1985) and Mls^{c} (Ryan et al. 1987a) specificities in a primary MLR. In addition, exposure of mouse splenocytes to heterologous (Ryan et al. 1983, 1987a, 1987b, 1988) or monoclonal (Ryan et al. 1983) GaMD in vivo for 24 h can substantially augment the presentation of both the Mls^{a} and Mls^{c} moieties that these cells express. Accordingly, prototypic Mls^{a} AKR/J, Mls^{b} CBA/CaJ, Mls^{c} C3H/HeJ, Mls^{a,c} CBA/J, and Mls^{null} CBA/N normal splenocytes were either irradiated or mitomycin C-treated and cultured with Mls^{b} B10.BR T cells in an Mls-defined MLR, Mls^{a,c} H-2^{d} (BALB/c × DBA/2)F_1 T cells in an H-2-defined MLR, and C58/J T cells in a presumably non-H-2-defined MLR (Table 2, experiment 1). As previously reported (Webb et al. 1985; Ryan et al. 1987a) for an Mls-defined MLR, irradiation substantially diminished the capacity of strongly-stimulatory AKR/J and CBA/J, as well as more poorly stimulatory C3H/HeJ splenocytes, to stimulate proliferation of H-2-compatible Mls^{b} B10.BR responder cells. The stimulatory ability of these splenocytes to present their MHC determinants was also noticably impaired after irradiation but the diminished level was never equivalent to that detected across a known Mls barrier. Like B10.BR responder cells, the capacity of C58/J T cells to respond was more greatly impaired after irradiation (versus mitomycin C-treatment) of the H-2^{k}-compatible presenting cells than was the proliferation of the MHC-disparate BALB/c F_1 T cells.

As expected, the prototypic Mls^{b} CBA/CaJ and Mls^{null} CBA/N mitomycin C-treated stimulator cells failed to stimulate effective levels of proliferation of Mls^{b} B10.BR T cells in an Mls-defined MLR. The finding that C58/J T cells proliferated to these splenocytes, which were formerly considered virtually nonstimulatory across a non-MHC barrier, was provocative (Table 2, experiment 1). Those splenocytes that were the most capable after mitomycin C treatment of eliciting high levels of DNA synthesis by B10.BR or C58/J responder cells were also the most mitogenic for these T cells when the presenting cells were pretreated with GaMD in vivo and then irradiated (Table 2, experiment 2). GaMD-treated splenocytes showed a peak in augmented presentation of this newly-defined non-MHC specificity one day after in vivo administration of this B cell mitogen; stimulatory capacity then diminished to control values seven days after injection of GaMD (data not shown). Prototypic Mls determinants show a similar time course in stimulating ability after in vivo GaMD treatment (Ryan et al. 1983).

C58/J T cells recognize a non-MHC MLR stimulatory specificity that is widely distributed among H-2^{k}-compatible mouse strains. Although C58/J mice have been assumed to be totally H-2-compatible with other H-2^{k} mouse strains, the possibility exists that C58/J may express an aberrant MHC; thus, the response of C58/J T cells to the Mls prototypic stimulator cells seen previously could be directed to their "conventional" MHC-associated antigens. Alternatively, C58/J T cells may be recognizing a shared non-MHC (perhaps Mls-like) moiety expressed by each of these Mls prototypic splenic stimulator cells. Indeed, the extreme radiation sensitivity and GaMD augmentability (Table 2) might argue for the latter possibility.

To distinguish formally between these two alternatives, C58/J mice were crossed with congenic *H-2^{k}* B10.BR, *H-2^{d}* B10.D2, and *H-2^{b}* C57BL/10 mice. If the C58/J T cell response was directed at an H-2^{k} component of the Mls prototypic stimulator cells, only the (B10.BR × C58/J)F_1 responder cells should be deleted of responsiveness to these H-2^{k} stimulator cells. If C58/J responder cells recognize a non-MHC component present in the background of each of the MHC-disparate B10 congenic strains (and shared with the Mls prototypic strains), the T cells from (B10.BR × C58/J)F_1, (B10.D2 × C58/J)F_1, and (C57BL/10 × C58/J)F_1 animals should all be nonresponsive to this stimulator panel. In the four experiments compiled in Table 3, additional H-2^{k} Mls non-prototypic stimulator cells were included to obtain a more comprehensive appreciation of the strain distribution of the non-MHC specificity recognized by C58/J. Because each of the three H-2-different B10 congenic F_1 responder cell populations was largely unresponsive to the Mls prototypic stimulator cells as well as the other H-2^{k}-compatible stimulator cells tested, it is likely that C58/J T cells recognize in a primary MLR a widely-distributed, non-MHC moiety shared by most of the H-2^{k} splenocytes examined. The ability of parental H-2^{d}, Mls^{a,c} DBA/2 to delete this responsiveness in (DBA/2 × C58/J)F_1 animals suggests that this Mls^{a} pro-

Table 3. C58/J responder cells recognize a non-MHC specificity that is widely-distributed among H-2^k-compatible inbred mice.

Stimulator cells*			Responder cells†				
Strain	H-2	Mls	C58/J	(B10.BR×C58)F$_1$	(B10.D2×C58)F$_1$	(C57BL/10×C58)F$_1$	(DBA/2×C58)F$_1$
Syngeneic			6,141‡	7,938	5,198	3,691	5,926
AKR/J	k	a	60,064	4,445	4,325	1,471	4,625
MA/My	k	a	174,665	23,112	24,644		
B10.BR	k	b	13,590	3,459	2,540	1,606	
CBA/CaJ	k	b	209,348	6,336	3,451	4,549	4,627
C57BR/cdJ	k	b	187,480	14,633	15,255		
B6.AKR	k	b	104,662	9,156	10,269		
C3H/HeJ	k	c	31,166	5,622	3,098	2,093	3,826
BALB.K	k	c	54,923	3,920	2,969	2,185	
CBA/J	k	a,c	144,950	7,271	4,306	4,631	4,651
CE/J	k	a,c	125,868	10,936	11,738	4,196	5,467
RF/J	k	a,c	6,838	3,915	4,422		2,073
C57BL/10	b	b	60,100	92,418	57,484		
B10.D2	d	b	109,256			76,559	
SJL/J	s	?	43,408				38,082

* Stimulator cells were splenocytes (6×10^5/well) that were irradiated with 3000 R after being obtained from animals that were injected 24 h beforehand with 100 μg of GaMD i.v.

† See legend to Table 1.

‡ See legend to Table 1; the cpm values represent the averages of four separate experiments.

totypic mouse strain also encodes the non-MHC ligand shared by the H-2^k mouse strains tested.

The large variation in the capacity of stimulator cells from different H-2^k mouse strains to trigger C58/J T cells across this non-MHC difference was also an important feature of these experiments. A gradient of stimulatory ability was noted, with B10.BR being among the poorest and CBA/CaJ, CBA/J, and C57BR/cdJ strains being among the best presenters of this non-MHC moiety. This laboratory previously reported that a diversity in presenting capacity also existed for the Mls^c specificity among different H-2^k mouse strains; this phenomenon reflected the influence of non-MHC background genes encoded by the stimulator cells (Ryan et al. 1990a). One additional example of the non-MHC regulated presentation of this newly-defined specificity may involve the B6 and B10 non-MHC backgrounds (both Mls^b) that were formerly considered to be quite similar. It was interesting to find that in this, as well as in other experiments not shown, the B6.AKR stimulator cells were superior in comparison with B10.BR splenocytes for the presentation of this non-H-2 MLR stimulatory determinant to C58/J T cells. The only H-2$_k$-compatible stimulator cells tested that did not elicit detectable levels of C58/J responder proliferation (in this and other experiments) were those from RF/J mice. Because Mls^{a,c} RF/J T cells are not responsive to Mls^a, Mls^c (Ryan et al. 1990a), or Mls^b (J.J. Ryan, unpublished observation) H-2^k-compatible stimulator cells, there is no evidence to suggest that RF/J responder cells also recognize the newly-defined, widely-distributed non-MHC moiety that stimulates C58/J T cells. Thus it is possible than spleen cells from RF/J mice encode this specificity but are not able to present this determinant effectively in vitro. We attempted to determine whether the RF/J parent was capable of deleting responsiveness in the (RF/J×C58/J)F$_1$ animal to this non-MHC specificity recognized by C58/J T cells. However, after more than one year, we did not obtain productive matings between these parental strains.

The largest residual response of (B10.BR×C58/J)F$_1$ and (B10.D2×C58/J)F$_1$ T cells to the panel of H-2^k stimulator cells was directed to the MA/My splenocytes. This could relate to the previous report that MA/My expresses a weak, non-MHC MLR stimulatory determinant, distinct from Mls^a and Mls^c (Ryan et al. 1990a). It is possible that this MA/My determinant is not a self-component of C58/J or the B10 congenic mice and is therefore responsible for eliciting the low level of residual F$_1$ responder proliferation detected in the MLR shown in Table 3.

Having shown that Mls^{null} CBA/N splenocytes also stimulate C58/J responder cells (Table 2, experiment 1), we wished to determine whether the specificity recognized on these xid$^+$ stimulator cells was similar to the widely-distributed, non-MHC determinant discussed above. Mitomycin C was used to inactivate normal splenic stimulator cells in this experiment, since we previously observed that GaMD-treated irradiated CBA/N splenocytes were not particularly effective in presenting this non-MHC specificity to C58/J T cells, unlike B-cell normal stimulator cells (Table 2, experiment 2). Accordingly, the response of C58/J T cells to mitomycin C-treated

Table 4. Although formerly considered Mls[null], CBA/N splenocytes express an MLR stimulatory non-MHC specificity recognized by C58/J responder cells that is shared with other H-2[k] Mouse Strains.

Stimulator cells*			Responder cells‡		
Strain	H-2	Mls	C58/J	CBA/CaJ	(CBA/CaJ × C58/J)F$_1$
Syngeneic	k	–	9,490#	1,720	2,179
CBA/J	k	a,c	51,071	194,281	3,395
CBA/N	k	null	39,280	3,162	9,648
B10.Q	q	b	82,634	67,228	82,721

* See legend to Table 1.
‡ See legend to Table 1.
#Values are cpm of mean arithmetic [^{3}H]TdR uptake of quadruplicate cultures, harvested at 96 h. *Underlined numbers* are those that are significantly greater ($p<0.05$) than response to syngeneic stimulators.

Table 5. Monoclonal antibody to class II MHC inhibits the response to the non-MHC specificity recognized by C58/J T cells.

Responder cells‡	Antibody	Stimulator cells*			
		C58/J	CBA/CaJ	CBA/J	B10.D2
Experiment 1					
C58/J	Medium	15,527#	104,438	56,192	179,483
	10-362	13,574	28,372	19,594	142,571
Experiment 2					
C58/J	Medium	5,299	47,055	45,753	117,798
	14-4-4	7,577	8,782	13,381	102,602
	MK-D6	6,294	46,498	32,801	48,931
	CBPC-101	9,449	48,496	44,275	128,749

* See legend to Table 1.
‡ See legend to Table 1.
#See legend to Table 4; the monoclonal 10–362 (A^k-specific) antibody (50 μg/ml) and 14-4-4 (E-specific) antibody (8 μg/ml) were purified from ascites fluid by ammonium sulfate precipitation and Sephadex colum chromatography; the monoclonal MK-D6 (A^d-specific) antibody (40 μg ml) and CBPC-101 (no known specificity) antibody (10 μg/ml) were purified by Protein A-Sepharose column chromatography.

CBA/N stimulator cells was compared to that of (CBA/CaJ × C58/J)F$_1$ T cells (Table 4). Crossing C58/J with CBA/CaJ was previously shown to produce an F$_1$ animal that is unresponsive to each of the H-2[k] stimulator cells listed in Table 3. Similarly, in this experiment the F$_1$ T cells were largely deleted of responsiveness not only to H-2[k]-compatible CBA/J, but also to CBA/N splenic stimulator cells. Because some significant residual proliferation of the (CBA/CaJ × C58/J)F$_1$ responder cells to CBA/N stimulator cells was noted in this and other experiments not shown, it is possible that CBA/N splenocytes also express an additional non-MHC determinant not shared with CBA/CaJ or CBA/J that stimulates C58/J T cells.

The finding that the F$_1$ responder cells were unresponsive to CBA/J stimulator cells, while parental CBA/CaJ and C58/J proliferated to these splenocytes is not inconsistent. It supports instead the concept of gene complementation in which the CBA/CaJ partner contributed the newly-defined, non-MHC stimulatory specificity and the C58/J partner contributed the prototypic Mls[a] and Mls[c] to yield an F$_1$ animal depleted of T cells that could respond to these three specificities expressed on H-2-compatible CBA/J stimulator cells. In summary, it is most likely that all H-2[k] strains tested (with the possible exception of RF/J) share the widely-distributed, non-MHC MLR specificity recognized by C58/J heterogenous T cells.

Monoclonal anti-Ia antibody blocks the responsiveness of C58/J T cells to the widely-distributed, non-MHC MLR stimulatory specificity. Studies from other laboratories have consistently demonstrated that monoclonal or polyclonal antibodies to MHC class II subregion antigens A or E block the proliferation of responsive T cells across an Mls[a] (Janeway et al. 1980; Macphail and Stutman 1984; Janeway and Katz 1985) and an Mls[c] (Abe and Hodes 1988) barrier. Therefore, we wished to test the effect of monoclonal Ia-specific antibodies on the recognition of the widely-distributed (non-Mls[a,c]), non-MHC MLR stimulatory specificity recognized by C58/J T cells. Accordingly, monoclonal 10-362 A^k- (Table 5, experi-

ment 1) or 14-4-4 E- (Table 5, experiment 2) specific antibody was added to the primary MLR between C58/J T cells and H-2-compatible mitomycin C-treated CBA/CaJ and CBA/J or H-2-disparate B10.D2 splenic stimulator cells. Very substantial blocking of C58/J responsiveness to the non-H-2 specificity presented by the H-2^k stimulator cells was observed with the addition of each of these monoclonal antibodies; however, much less effect was noted on the proliferation of C58/J T cells to H-2^d-disparate B10.D2 splenocytes. MK-D6 A^d-specific monoclonal antibody had only a small effect on the response of C58/J T cells to the newly-defined, non-MHC specificity expressed by H-2^k-compatible CBA/CaJ and CBA/J; however, a substantial reduction in the C58/J T cell response to H-2^d B10.D2 was observed. The CBPC-101 antibody (with no known antigen specificity) has an IgG_{2a} isotype like 10-362, 14-4-4, and MK-D6. This isotype-matched control reagent had no effect on the anti-non-MHC or anti-MHC response of C58/J T cells in a primary MLR. Thus, as with prototypic Mls^a and Mls^c, class II MHC molecules influence the recognition of this newly-defined, non-MHC MLR stimulatory specificity and specific antibodies to Ia antigen prevent effective presentation/recognition of this moiety. In a preliminary experiment, monoclonal anti-H-2K^k antibody 11-4.1 also reduced the C58/J T cell proliferative response to the newly-defined, non-MHC determinant (data not shown). This is compatible with the previous report that an anti-MHC class I antibody partially blocked responder T cell proliferation to prototypic Mls^a (Macphail and Stutman 1984).

Phenotypically normal CBA/N F_1 female but not xid$^+$ B cell-defective CBA/N F_1 male stimulator cells effectively present the newly-defined, non-MHC MLR stimulatory specificity recognized by C58/J T cells. For both the Mls^a (Ryan et al. 1983; Webb et al. 1984) and the Mls^c (Ahmed and Scher 1976; Ryan et al. 1990a) specificities, B cell-defective xid$^+$ CBA/N F_1 male splenic stimulator cells are poor presenters of these non-MHC moieties while B cell-normal CBA/N F_1 female splenocytes more effectively stimulate responder T cells across these barriers. Substantial differences in the ability to present MHC determinants have not been observed for CBA/N F_1 male and female stimulator cells (Webb et al. 1984). To examine the functional similarities and differences between prototypic Mls antigens and the newly-defined, non-MHC MLR stimulatory specificity further, the capacity of splenocytes from CBA/N F_1 male and female animals to present the latter determinant and prototypic Mls^a in a primary MLR was compared (Table 6).

Because the frequency of Ig-positive spleen cells in xid$^+$ CBA/N F_1 male mice is approximately 40% less than that found in their phenotypically B cell-normal female F_1 littermates (Scher et al. 1975), male and female CBA/N F_1 stimulator cells were T cell-depleted (before mitomycin C treatment) to obtain approximately equivalent numbers of non-T Mls presenting cells in both groups. Consistent with previous reports (Ryan et al. 1983; Webb et al. 1984), C3H/HeJ T cells proliferated vigorously to the Mls^a difference expressed by phenotypically normal female but not xid$^+$ male (CBA/N × AKR/J)F_1 splenic stimulator cells (Table 6, experiment 1). Interestingly, although C58/J animals encode Mls^a (Abromson-Leeman et al. 1988a, 1988b; Ryan et al. 1990a) and are deleted of T cells that express the Vβ segments that are predictive of responsiveness to Mls^a (Vacchio and Hodes 1989), C58/J responder cells also proliferated vigorously to the (CBA/N × AKR/J)F_1 female but not male stimulator cells. Based on the results of Tables 3 and 4, it is likely that both CBA/N and AKR/J partners contribute to their F_1 the widely-distributed non-MHC MLR stimulatory specificity, distinct from prototypic Mls antigens, recognized by C58/J.

In the next series of experiments, Mls^{null} CBA/N mice were crossed with H-2^k-compatible animals, Mls^b B10.BR and CBA/CaJ, that were not known to express a stimulatory (previously defined) Mls specificity. The latter prototypic Mls nonstimulatory strains nevertheless expressed the widely-distributed, non-MHC MLR stimulatory specificity recognized by C58/J T cells (Table 3). The resulting normal female and defective xid$^+$ male F_1 splenocytes were examined for a differential capacity to present this newly-defined non-MHC MLR stimulatory specificity to C58/J T cells (Table 6, experiments 2 and 3). As with the presentation of prototypic Mls^a by (CBA/N × AKR/J)F_1 splenocytes (Table 6, experiment 1), the non-MHC specificity recognized by unprimed C58/J responder cells was more effectively presented by stimulator cells from B cell-normal (CBA/N × B10.BR) F_1 female than the xid$^+$ F_1 male littermates. A comparable result was obtained with normal mitomycin C-treated (CBA/N × CBA/CaJ)F_1 male and female splenocytes.

The response of C58/J T cells to H-2^k-compatible parental xid$^+$ CBA/N splenocytes (observed in Tables 1 and 4) appeared to be somewhat more vigorous than to the xid$^+$ CBA/N F_1 male stimulator cells detected in Table 6. This could be explained in part by the previous observation that CBA/N splenocytes may express an additional non-MHC specificity stimulatory for C58/J T cells (Table 4); this determinant, that is not extremely stimulatory when presented by homozygous CBA/N, might be undetected in the heterozygous CBA/N F_1 male splenocytes. Nevertheless, parental xid$^+$ CBA/N splenocytes were less stimulatory for C58/J T cells than the phenotypically B cell-normal CBA/N F_1 female splenocytes (Table 6, experiment 2) and presenting cells from CBA/CaJ and CBA/J (Tables 2 and 4) mice to which the immunodeficient CBA/N strain is related. The non-MHC

Table 6. The non-MHC specificity recognized by C58/J T cells is well presented by phenotypically normal CBA/N F_1 female but not XID$^+$ CBA/N F_1 male stimulator cells.

Stimulator cells*				Responder cells‡	
Strain	Mls	GaMD treatment	Cell no. $\times 10^{-5}$	(Mls^a MLR) C3H/HeJ	(Non-H-2 MLR) C58/J
Experiment 1					
Syngeneic	–	–	3	2.509≠	1.551
			6	3.054	2.638
(CBA/N×AKR)F_1 female	null, a	–	3	138.062	47.431
			6	141.791	65.657
(CBA/N×AKR)F_1 male	null, a	–	3	13.360	2.988
			6	19.531	3.519
Experiment 2				(Non-H-2 MLR) C58/J	(H-2 MLR) B6D2F_1
Syngeneic	–	–	3	8.586	4.526
			6	9.327	
(CBA/N×B10.BR)F_1 female	null, b	–	3	58.619	82.223
			6	77.403	
(CBA/N×B10.BR)F_1 male	null, b	–	3	10.549	61.871
			6	11.293	
CBA/N	null	–	3	23.748	54.233
Experiment 3				(Non-H-2 MLR) C58/J	(H-2 MLR) BALB/C
Syngeneic	–	–	3	4.071	3.566
			6	1.573	3.384
		+	3	5.844	3.512
			6	4.574	3.093
(CBA/N×CBA/CaJ)F_1 female	null, b	–	3	36.339	39.909
			6	48.465	35.001
		+	3	190.127	48.328
			6	158.159	30.226
(CBA/N×CBA/CaJ)F_1 male	null, b	–	3	4.213	32.039
			6	5.550	28.932
		+	3	17.831	30.886
			6	15.619	18.944

* Stimulator cells were normal or GaMD-activated splenocytes that were treated with anti-Thy 1.2+C and then mitomycin C-treated (Experiments 2, 3) or normal splenocytes treated with anti-Thy 1.1+anti-Thy 1.2+C and then mitomycin C-treated (Experiment 1).
‡ See legend to Table 1.
≠ See legend to Table 1.

stimulatory capacity of the (CBA/N×CBA/Ca)F_1 female splenocytes for C58/J responder cells was substantially enhanced after GaMD treatment (Table 6, experiment 3). In contrast, the xid+ GaMD-activated (CBA/N×CBA/Ca)F_1 male splenocytes showed a modest increase in stimulatory ability across this non-MHC barrier. The latter observation does not necessarily conflict with our original report that GaMD treatment of xid$^+$ CBA/N F_1 male stimulator cells had no effect on the presentation of prototypic Mls antigen (Ryan et al. 1983). In that study, the stimulator cells were irradiated while in these experiments, the presenting cells in the MLR were mitomycin C-treated. Given the extreme radiation sensitivity of B cells with the xid$^+$ phenotype (Riggs et al. 1988), the Mls augmenting effect of GaMD pretreatment might not be detected subsequent to a high dose of irradiation given to xid$^-$ stimulator cells in the earlier study.

Discussion

For many years, a strong Mls antigen was considered an in vitro curiosity that could trigger the explosive proliferation of H-2-compatible responder cells in a primary MLR due to the large precursor frequency of T cells that were precommitted to recognize this non-MHC moiety. More recently, however, Mls^a and Mls^c determinants have been recognized to belong to a class of "superantigens" that play an important role in the elimination of self-reactive T cells during development in the thymus (Kappler

et al. 1988; MacDonald et al. 1988; Pullen et al. 1988). In addition, studies of the depletion of mature T cells expressing Vβ5.2$^+$ (Woodland et al. 1990), Vβ7$^+$ (Vacchio and Hodes 1989), Vβ11$^+$ (Bill et al. 1989; Vacchio and Hodes 1989), or Vβ17a$^+$ (Marrack and Kappler 1988) Tcr segments in mice bearing a class II EαEβ product suggested that undefined non-MHC self-deleting ligands besides Mls^a and Mls^c could exist.

In a comprehensive investigation of the range of self-antigens that influence Vβ usage, it was reported that C58/J animals, unlike other E$^+$ mouse strains, were not deleted of Vβ11$^+$ or Vβ12$^+$ T cells (Vacchio and Hodes 1989). These authors further determined that non-MHC ligands, not encoded in C58/J animals, mediate the deletion of Vβ11$^+$ or Vβ12$^+$ T cells in the periphery of other inbred strains (Vacchio et al. 1990). In addition, this laboratory has found that Mls^{a,c} C58/J T cells responded to H-2-compatible Mls^a AKR/J and Mls^c C3H/HeJ stimulator cells in a primary MLR (Ryan et al. 1990a). These observations were in agreement with the limited MLR data included in another study (Vacchio et al. 1990) of the negative selection of Vβ11- and Vβ12-expressing T cells. However, there remains an additional need to determine whether unique allostimulatory characteristics commonly associated with prototypic Mls^a and Mls^c are shared with non-MHC antigen recognized by C58/J T cells in a primary MLR.

Before addressing the nature of the specificity recognized by C58/J T cells in a non-MHC-defined MLR, it is first necessary to establish firmly that C58/J animals are genotypically Mls^{a,c} and thus self-tolerant to these prototypic Mls determinants. In this regard, both Mls^a or Mls^c-specific T cell clones proliferated to C58/J splenic stimulator cells (Abromson-Leeman et al. 1988a, 1988b). In a primary MLR, H-2-compatible Mls^a AKR/J and Mls^c C3H/HeJ but not Mls^{a,c} (AKR/J $\times$ C3H/HeJ)F$_1$ (Ryan et al. 1990a) or CBA/J (Fig. 1) T cells responded to C58/J stimulator cells. Parental Mls^{a,c} prototypic CBA/J mice when crossed with Mls^b B10.BR mice induced unresponsiveness in the (B10.BR $\times$ CBA/J)F$_1$ progeny not only to H-2-compatible Mls^a prototypic AKR/J, Mls^c prototypic C3H/HeJ, and Mls^{a,c} (AKR/J $\times$ C3H/HeJ)F$_1$ (Ryan et al. 1987b; Table 1) but also to C58/J stimulator cells. In addition, C58/J animals are depleted of T cells that express Vβ6 and Vβ9 Tcr segments, that are associated with responsiveness to Mls^a, as well as Vβ3 Tcr segments, and that are associated with responsiveness to Mls^c (Vacchio and Hodes 1989). Taken together, these observations indicate that proliferation across a non-MHC difference by C58/J responder cells would be directed to a moiety other than Mls^a or Mls^c.

A related issue to resolve at the outset was the possibility that C58/J mice express aberrant MHC antigen, so that the proliferative response of C58/J T cells to Mls^a, Mls^b, Mls^c, or Mls^{a,c} stimulator cells (that are supposedly H-2-compatible with C58/J) would directed at their conventional H-2^k MHC determinants. This possibility was excluded by generating a series of F$_1$ responders by crossing C58/J animals with H-2^k B10.BR, H-2^d B10.D2, or H-2^b C57BL/10 mice. If the response of C58/J T cells to the H-2^k stimulator cells tested (Table 3) was actually directed at their MHC-encoded determinants, only the (B10.BR $\times$ C58/J)F$_1$ mice among these F$_1$ responders would be self-tolerant to this panel of stimulators. Because the (B10.D2 $\times$ C58/J)F$_1$ and (C57BL/10 $\times$ C58/J)F$_1$, as well as the (B10.BR $\times$ C58/J)F$_1$ T cells were no longer responsive to these H-2^k stimulator cells (Table 3), we concluded that C58/J responder cells recognize a non-MHC ligand, distinct from previously defined Mls^a or Mls^c, that is present in the B10 congenic background and is widely distributed among other inbred mouse strains.

Subsequently, we analyzed additional functional properties of the non-MHC moiety recognized by C58/J T cells in a primary MLR to determine if it had characteristics common to established members of the Mls superantigen family. Since no biochemical or serological criteria exist that characterize prototypic Mls determinants, the decision whether a newly-defined, non-MHC MLR stimulatory specificity belongs to this family of superantigens must be based largely on functional evidence. Taken together, our observations that the non-MHC MLR stimulatory specificity recognized by heterogenous C58/J T cells (like prototypic Mls^a and Mls^c), is particularly radiation sensitive, is dramatically augmented after stimulator cell GaMD treatment and "triggers high levels of T cell proliferation, is influenced in its presenting capacity by non-MHC stimulator background genes, is blocked with anti-class II MHC antibody, and is presented much more effectively by B cell-normal female than by B cell-defective xid$^+$ male CBA/N F$_1$ splenic stimulator cells strongly suggest that this moiety should be considered a new member of the Mls superantigen family. Upon examination of these functional properties, the B lymphocyte appears to play as prominent a role in the presentation of the newly-defined, non-MHC MLR stimulatory specificity as was previously documented for prototypic Mls determinants (Webb et al. 1985; Ryan et al. 1988) and at least one undefined superantigen (Marrack and Kappler 1988).

Because unresponsiveness to the newly-defined, non-MHC MLR stimulatory determinant is dominant in F$_1$ animals obtained by crossing C58/J mice with mouse strains that encode this non-MHC antigen (Tables 1, 3, and 4), it is likely that this ligand mediates in vivo the negative selection of T cell populations that are self-reactive to it. This functional observation compliments the finding that deletion of Vβ11$^+$ or Vβ12$^+$ T cells is dominant in F$_1$ mice obtained by crossing (Vβ11$^-$ Vβ12$^-$) strains that express this newly-defined, non-

MHC determinant and (Vβ11$^+$ Vβ12$^+$) parental C58/J (Vacchio et al. 1990). This self-deleting property is now considered a hallmark of prototypic Mls antigens (Abe et al. 1988; Kappler et al. 1988; MacDonald et al. 1988; Pullen et al. 1988; Happ et al. 1989; Pullen et al. 1989; Vacchio and Hodes 1989).

Although a low level of C58/J T cell proliferation was elicited across this non-MHC barrier by B10.BR splenocytes, (B10.BR × C58/J)F_1 animals were effectively deleted of functional responsiveness to this newly-defined specificity (Table 3). In addition, crossing C58/J with B10 congenic mice yielded F_1 animals that lacked Vβ11$^+$ or Vβ12$^+$ T cells in their periphery (Vacchio et al. 1990). Therefore, it is possible that the efficiency with which this non-MHC determinant evokes the negative clonal selection process in vivo for a particular inbred mouse strain may not correlate with its potency as a T cell-stimulatory antigen in vitro. The congenic B10.BR strain derived its H-2^k haplotype from C57BR/cd mice that very effectively present this newly-defined, non-MHC MLR stimulatory specificity recognized by C58/J T cells (Table 3). Consequently, it is likely that the action of non-*MHC* genes present in the B10 background rather than the expression of an "inappropriate" or aberrant MHC accounts for the poor in vitro presentation of this non-MHC MLR stimulatory specificity by B10.BR splenocytes. Previous studies from this laboratory have confirmed the importance of non-*MHC* gene influences in regulating the presentation of prototypic Mls^a and Mls^c determinants (Ryan et al. 1990a).

The finding that monoclonal E-specific antibody blocks responsiveness of C58/J T cells to this MLR stimulatory non-MHC antigen (Table 5, Experiment 2), is consistent with other studies indicating that expression of the E molecule is critical for self-recognition of a widely-distributed, non-MHC moiety and subsequent clonal deletion of self-reactive T cells that are Vβ11$^+$ (Bill et al. 1989; Vacchio and Hodes 1989). The reason why monoclonal A-specific antibody is as effective as E-specific antibody in eliminating proliferation of heterogenous C58/J T cells to this ligand that may be co-recognized with the class II E molecule is not immediately obvious. However, it is important to emphasize that mouse T cell responsiveness to the prototypic Mls^c determinant, that is clearly restricted by the E molecule of the stimulator cells (Ryan et al. 1987; Abe and Hodes 1988), is also blocked by monoclonal E- and A-specific antibodies (Abe and Hodes 1988).

The importance of the MHC in the presentation of previously defined Mls specificities has been carefully documented by several laboratories (Peck et al. 1977; Lynch et al. 1985; Macphail and Stutman 1986; Ryan et al. 1987a; Abe and Hodes 1988): H-2-different congenic and recombinant mouse strains have often been employed as stimulator cells in those studies to map the critical *H-2* subregion that is co-recognized with the prototypic Mls determinant. However, the extremely wide distribution of the newly-defined, non-MHC MLR stimulatory specificity among inbred mice may make this type of immunogenetic analysis for this antigen difficult with unprimed heterogenous responder T cells. For example, to generate appropriate F_1 responder cells tolerant to allogeneic MHC yet potentially responsive to the newly-defined, non-MHC determinant, C58/J mice should be crossed with H-2-disparate mouse strains that do not encode this non-MHC antigen as a self-component. Since most inbred strains tested (Tables 3 and 4) – except C58/J – encode this non-MHC determinant, finding such an H-2-different partner lacking the non-MHC antigen may be difficult.

Furthermore, backcross analysis experiments that would attempt to determine whether the newly-defined, non-MHC MLR stimulatory determinant(s) segregates independently of prototypic Mls^a or Mls^c antigen requires that one of the parental strains does not encode the former specificity. Thus, the widespread distribution of this determinant complicates segregation analysis as well. In addition, two or more genes were shown to encode this non-MHC ligand(s) based on the deletion of Vβ11$^+$ T cells in (CBA/Ca × C58/J)F_1 × C58/J first backcross animals (Vacchio et al. 1990). Given that multiple genes also encode Mls^c (Click and Adelman 1988; Abe et al. 1989; Pullen et al. 1989) and possibly Mls^a (Click et al. 1982; Ryan et al. 1990b), evaluation of allelism between this newly-defined, non-MHC specificity or specificities and previously-defined *Mls* gene products would be an extremely difficult immunogenetic exercise. However, the observation that this newly-defined, non-MHC determinant is simultaneously expressed by Mls^{a,c} mouse strains (e.g., CBA/J and CE/J; Table 3), suggests, but does not prove, that it is not an allele of at least some of the gene products associated with the Mls^a or Mls^c phenomena. Clearly, cloned T cells specific for the non-H-2 MLR stimulatory determinant recognized by C58/J responder cells must be derived in order to address effectively the issues of MHC restriction and of possible allelic relationships with previously defined Mls antigens.

The Mls^a and Mls^c specificities were shown to be distinct nonallelic unlinked moieties that segregate independently of one another (Abe et al. 1987a). The Mls^b designation was used classically to indicate the absence of Mls^a and Mls^c on presenting cells and thus their nonstimulatory nature in a non-H-2-defined MLR (Festenstein 1976). The Mls^d determinant was demonstrated to represent the simultaneous expression of Mls^a and Mls^c antigens (Abe et al. 1987b; Ryan et al. 1987b). Mls^e was assigned in one report to a non-MHC MLR stimulatory determinant expressed by C3H/Tif mice that was presumably distinct from Mls^a and Mls^c (Coutinho et al. 1977). The original small case letter nomenclature,

as opposed to the revised numerical designations (Abromson-Leeman et al. 1988a; Janeway et al. 1989), offers the advantage of providing collective symbols that can be used to convey correctly the multigenic nature (Click et al. 1982; Click and Adelman 1988; Abe et al. 1989; Pullen et al. 1989; Ryan et al. 1990b) of the known Mls determinants. Because Vβ usage and deletion studies suggest that multiple genes encode the non-MHC ligand(s) recognized by C58/J T cells (Vacchio et al. 1990), the use of a letter designation to label this moiety also seems appropriate. Thus we propose that the latter newly-defined and widely-distributed non-MHC MLR stimulatory determinant(s), that shares very similar functional allostimulatory properties with prototypic Mls^a and Mls^c, be assigned the Mls^f designation.

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